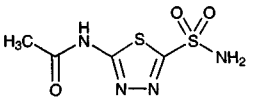
Acetazolamide



Molecular formula: $C_4H_6N_4O_3S_2$
Molecular weight: 222.25
CAS Registry No.: 59-66-5, 1424-27-7 (sodium salt)
Merck Index: 50
Lednicer No.: 1 249, 1 111

SAMPLE

Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM

Retention time: 5.8
Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordoxepin, nordiazepam, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trazodone, verapamil
Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin
Interfering: amitriptyline, norfluoxetine, trimipramine

KEY WORDS

plasma; SPE

REFERENCE
Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE
Matrix: blood, CSF
Sample preparation: 200 μL Serum, plasma, or CSF + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES
Column: A 30 × 2.1 40 μm preparative grade C18 (Analytichem); B 250 × 4.6 10 μm Partisil C8
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 280 for 5 min then UV 254

CHROMATOGRAM
Retention time: 4.89
Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES
Extracted: bromazepam, caffeine, carbamazepine, chloramphenicol, chlorothiazide, diazepam, droperidol, ethionamide, furosemide, isoniazid, methadone, penicillin G, phenobarbital, phenytoin, prazepam, propoxyphene, pyrazinamide, rifampin, trimeprazine, trimethoprim
Interfering: ampicillin

KEY WORDS
serum; plasma; column-switching

REFERENCE
Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

SAMPLE
Matrix: blood, urine
Sample preparation: To 500 μL whole blood, plasma, or urine add 2.5 mL 50% ammonium sulfamate, vortex for 30 s. (Place the tube containing whole blood in boiling water for 30 s and then quickly in cold water.) Add 5 mL ethyl acetate, vortex, centrifuge at 3000 g for 10 min, transfer the organic layer to 5 mL pH 8.0 phosphate buffer, vortex, centrifuge at 3000 g for 10 min, transfer the organic layer to 500 μL pH 10.0 glycine buffer, vortex for 30 s, centrifuge at 3000 g for 5 min. Aspirate and discard the organic layer, add 500 μL ether to the remaining glycine buffer layer, vortex for 1 min, discard the ether phase. Vent the aqueous layer for about 30 min and inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Altima C18 (Alltech)
Mobile phase: MeCN:100 mM pH 4.0 sodium acetate 20:80
Flow rate: 1
Injection volume: 20
Detector: 285

CHROMATOGRAM
Retention time: 4.55
Internal standard: acetazolamide

KEY WORDS
plasma; whole blood; acetazolamide is IS

REFERENCE
Iyer,G.R.; Taft,D.R. Determination of methazolamide concentrations in human biological fluids using high performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 1021–1027.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 265.3

CHROMATOGRAM
Retention time: 6.927

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: perfusate, urine
Sample preparation: Mix 350 μL perfusate or urine with 350 μL MeCN. Add 26 μL 1 mg/mL IS, vortex, centrifuge at 10000 g for 10 min, add 1 mL dichloromethane to 500 μL of the resultant supernatant, vortex, centrifuge at 10000 g for 20 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: Resolvex C18 (Fisher Scientific, Pittsburgh, PA)
Mobile phase: MeCN:100 mM pH 4.0 sodium acetate buffer 15:85
Flow rate: 1

Injection volume: 50

CHROMATOGRAM
Internal standard: sulfadiazine

KEY WORDS
rat; kidney

REFERENCE
Taft,D.R.; Chapron,D.J.; Fournier,D.J.; Sweeney,K.R. Concentration-dependent tubular secretion of acetazolamide and its inhibition by salicylic acid in the isolated perfused rat kidney, *Drug Metab.Dispos.*, **1996**, *24*, 456–461.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: μBondapak NH2
Mobile phase: MeOH:10 mM ammonium formate 20:80
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Limit of detection: 20 ng

KEY WORDS
rabbit; buffer

REFERENCE
Tang-Liu,D.D.-S.; Richman,J.B.; Weinkam,R.J.; Takruri,H. Effects of four penetration enhancers on corneal permeability of drugs in vitro, *J.Pharm.Sci.*, **1994**, *83*, 85–90.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 4.77 (A), 3.68 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem,

diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 µL 50 µg/mL β-hydroxyethyltheophylline in MeOH, inject 5 µL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 µm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)

Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 5

Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 4.30 (A); B (5.2)

Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, flumethiazide, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone

Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine

Sample preparation: Make 5 mL urine alkaline (pH 9-10), add 2 g NaCl, extract twice with 6 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN/water, inject a 10-20 μL aliquot.

HPLC VARIABLES

Column: 100 × 4 5 μm SGE 100 GL-4 C18P (Scientific Glass Engineering)

Mobile phase: MeCN:MeOH:water:trifluoroacetic acid 0.3:0.7:99:0.5

Flow rate: 0.8 or 1

Injection volume: 10-20

Detector: MS, ZAB2-SEQ (VG), PSP source coupled to LC, source 250°, probe 240-260°, scan m/z 200-550 or UV 270

CHROMATOGRAM

Retention time: 3.9

Limit of detection: <10 ng (by MS)

OTHER SUBSTANCES

Extracted: hydrochlorothiazide

REFERENCE

Ventura,R.; Fraisse,D.; Becchi,M.; Paisse,O.; Segura,J. Approach to the analysis of diuretics and masking agents by high-performance liquid chromatography-mass spectrometry in doping control, *J.Chromatogr.*, **1991**, *562*, 723–736.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g NaH_2PO_4:Na_2HPO_4 99:1 + 0.5 g NaCl (final pH 5-5.5), add 4 mL ethyl acetate, extract in a mechanical agitator for 10 min. Remove the organic layer and add it to 2 mL 5% lead acetate, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, filter (15 mm nylon Teknokroma 0.45 μm filter), inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 45 μm HP-Hypersil ODS-C18

Mobile phase: Gradient. MeCN:buffer from 12:88 to 15:85 over 3 min, to 40:60 after another 2 min, maintain at 40:60 (Buffer was 3.45 g $NaH_2PO_4.H_2O$ in 500 mL water and 0.7 mL propylamine hydrochloride, pH adjusted to 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 5

Detector: UV 275

CHROMATOGRAM
Retention time: 5.4
Internal standard: β-hydroxyethyltheophylline (4.6)
Limit of detection: 8 ng/mL

OTHER SUBSTANCES
Simultaneous: theobromine, theophylline, 1,7-dimethylxanthine, caffeine

REFERENCE
Herráez-Hernández,R.; Campíns-Falcó,P.; Sevillano-Cabeza,A. Determination of acetazolamide in human urine samples by reversed-phase high-performance liquid chromatography in the presence of xanthines, *J.Chromatogr.*, **1992**, *582*, 181–187.

SAMPLE
Matrix: urine
Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES
Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18
Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)
Flow rate: 1
Injection volume: 40
Detector: UV 270

CHROMATOGRAM
Retention time: 9.8
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: amiloride, bendroflumethiazide, benzthiazide, bumetanide, caffeine, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, probenecid, spironolactone, triamterene, trichlormethiazide

KEY WORDS
column-switching; optimum detection wavelengths vary for each drug

REFERENCE
Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

SAMPLE
Matrix: urine
Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN:water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: 2.2
Internal standard: 7-propyltheophylline (4.5)
Limit of detection: 2000 ng/mL

OTHER SUBSTANCES

Simultaneous: xipamide, bumetanide, amiloride, bendroflumethiazide, benzthiazide, buthiazide, caffeine, canrenone, chlorthalidone, clopamide, cyclothiazide, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, mesocarb, morazone, piretanide, polythiazide, probenecid, spironolactone, torsemide, triamterene

REFERENCE

Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

SAMPLE

Matrix: urine
Sample preparation: Inject a 5 μL aliquot directly onto column A with mobile phase A and elute to waste, after 0.3 min elute from column A onto column B with mobile phase A for 1.2 min then remove column A from the circuit and elute column B with mobile phase B, starting the gradient. Three minutes after the start change mobile phase A to MeCN:mobile phase A buffer 50:50 over 2 min to clean column A.

HPLC VARIABLES

Column: A 20 × 2.1 30 μm Hypersil ODS C18; B 125 × 4 5 μm HP-LiChrospher 100 RP 18
Mobile phase: A 3.45 g $NaH_2PO_4.H_2O$ in 500 mL water, pH adjusted to 3 with concentrated phosphoric acid; B Gradient. MeCN:water 0:100 for 2 min then to 50:50 over 2 min, maintain at 50:50
Injection volume: 5
Detector: UV 275

CHROMATOGRAM

Retention time: 5.27
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Noninterfering: caffeine, theophylline, theobromine, 1,7-dimethylxanthine

KEY WORDS

column-switching; heart-cut

REFERENCE

Campíns-Falcó,P.; Herráez-Hernández,R.; Sevillano-Cabeza,A. Application of column-switching techniques to the determination of medium polarity drugs: determination of acetazolamide in urine, *J.Chromatogr.B*, **1994**, *654*, 85–90.

SAMPLE

Matrix: urine

Sample preparation: Direct injection into column A with mobile phase A for 1 min then back flush onto column B with mobile phase B.

HPLC VARIABLES

Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 5 μm Hypersil ODS-C18

Mobile phase: A Water; B Gradient. MeCN:buffer 15:85 for 1.5 min then to 80:20 over 8 min. Keep at 80:20 for 2.5 min then re-equilibrate with 15:85. (Buffer was 50 mM NaH_2PO_4 + 1.4 mL propylamine hydrochloride per liter adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 50

Detector: UV 230

CHROMATOGRAM

Retention time: 5.4

Limit of detection: 40 ng/mL.

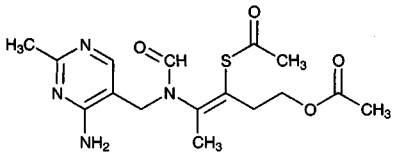
OTHER SUBSTANCES

Simultaneous: bumetanide, ethacrynic acid, amiloride, bendroflumethiazide, chlorthalidone, cyclothiazide, furosemide, hydrochlorothiazide, probenecid, spironolactone, triamterene

REFERENCE

Campíns-Falco,P.; Herráez-Hernández,R.; Sevillano-Cabeza,A. Column-switching techniques for screening of diuretics and probenecid in urine samples, *Anal.Chem.*, **1994**, *66*, 244–248.

Acetiamine



Molecular formula: $C_{16}H_{22}N_4O_4S$
Molecular weight: 366.44
CAS Registry No.: 299-89-8, 28008-04-0 (HCl)
Merck Index: 51

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 11.78

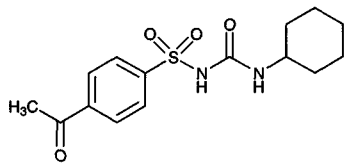
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Acetohexamide



Molecular formula: $C_{15}H_{20}N_2O_4S$
Molecular weight: 324.40
CAS Registry No.: 968-81-0
Merck Index: 59
Lednicer No.: 1 138

SAMPLE

Matrix: blood, urine
Sample preparation: Dilute urine 5 times with water. 200 μL Plasma or diluted urine + 2 mL 100 mM pH 5.0 phosphate buffer + 5 mL cyclohexamide in benzene:ethyl acetate 1:1 (Caution! Benzene is a carcinogen!), shake, centrifuge at 3000 rpm for 20 min. Remove 4 mL of the organic layer and evaporate it to dryness under vacuum at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5 C18
Mobile phase: MeCN:0.2% acetic acid 50:50
Flow rate: 1.2
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Internal standard: cyclohexamide

OTHER SUBSTANCES

Extracted: metabolites, hydroxyhexamide

KEY WORDS

plasma; rat; guinea pig; rabbit

REFERENCE

Asada,S.; Nagamine,S.; Nakae,H. Comparative pharmacokinetics of acetohexamide and its metabolite, hydroxyhexamide in laboratory animals, *Chem.Pharm.Bull.(Tokyo)*, **1989**, *37*, 760–765.

SAMPLE

Matrix: erythrocytes
Sample preparation: 1 mL erythrocyte mixture + 100 μL 10 μM tolazamide in MeOH, extract with toluene:ethyl acetate 1:1. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μL MeCN, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 3 μm Ultrasphere ODS
Mobile phase: MeCN:0.2% acetic acid 47:53
Flow rate: 1
Detector: UV 230

CHROMATOGRAM

Internal standard: tolazamide

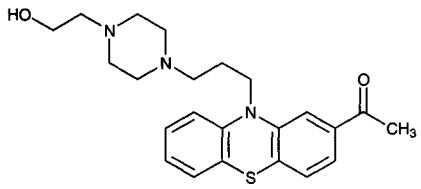
OTHER SUBSTANCES

Extracted: hydroxyhexamide

REFERENCE

Kishimoto,M.; Kawamori,R.; Kamada,T.; Inaba,T. Carbonyl reductase activity for acetohexamide in human erythrocytes, *Drug Metab.Dispos.*, **1994**, *22*, 367–370.

Acetophenazine



Molecular formula: $C_{23}H_{29}N_3O_2S$
Molecular weight: 411.57
CAS Registry No.: 2751-68-0, 5714-00-1 (dimaleate)
Merck Index: 70

SAMPLE

Matrix: blood
Sample preparation: 10 mL Plasma or whole blood + 1 mL 1 M NaOH, extract twice with 10 mL hexane for 30 min. Remove the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 100 mM HCl, add 5 mL chloroform, vortex for 1 min, centrifuge. Remove a 4.5 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. (It is implied, but not explicitly stated in the paper, that this extraction procedure works for this compound.)

HPLC VARIABLES

Column: 10 μm Micropak CN (Varian)
Mobile phase: MeCN:20 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 7.4
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: amitriptyline, benztropine, butaperazine, carphenazine, chlorpromazine, fluphenazine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, promazine, promethazine, thioridazine, thiothixene, trifluoperazine, trifluopromazine, trimeprazine
Interfering: trihexyphenidyl

KEY WORDS

plasma; whole blood

REFERENCE

Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.6

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 70:1.5:0.5:28

Flow rate: 1.5

Injection volume: 10

Detector: UV 254

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: mesoridazine, promethazine, chlorpromazine, thioridazine, prochlorperazine, butaperazine, thiethylperazine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl
Mobile phase: Carbon dioxide:MeOH:isopropylamine 90:10:0.05
Column temperature: 50
Flow rate: 3
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 4.33

OTHER SUBSTANCES
Simultaneous: trifluprom azine, carphenazine, methotrimeprazine, promazine, perphenazine, chlorprothixene, thiothixene, reserpine, ethopropazine, promethazine, propiomazine
Interfering: deserpidine

KEY WORDS
SFC; pressure 200 bar

REFERENCE
Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 1. Phenothiazine antipsychotics, *J.Pharm.Sci.*, **1994**, *83*, 281–286.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bi-

bucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25

Detector: UV 229

CHROMATOGRAM

Retention time: 9.83 (A), 4.89 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

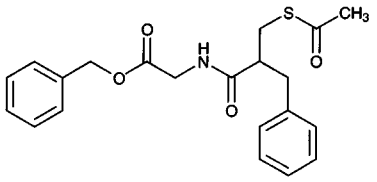
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Acetorphan



Molecular formula: $C_{21}H_{23}NO_4S$
Molecular weight: 385.48
CAS Registry No.: 81110-73-8
Merck Index: 72

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 22.495

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize kidney in 30 volumes of 50 mM pH 7.4 Tris-HCl buffer (Ultra-Turrax homogenizer), filter through a GF/B filter presoaked in 0.3% polyethylenimine, wash filter three times with 4 mL 50 mM pH 7.4 Tris-HCl buffer. Extract filter with 1 mL EtOH, centrifuge extract, inject an aliquot.

HPLC VARIABLES

Column: μBondapak C18
Mobile phase: MeCN:100 mM pH 6.2 ammonium acetate 70:30
Flow rate: 1
Detector: UV 210

OTHER SUBSTANCES

Extracted: metabolites, thiorphan

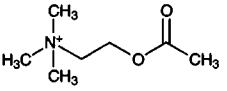
KEY WORDS

mouse; kidney

REFERENCE

De la Baume,S.; Brion,F.; Dam Trung Tuong,M.; Schwartz,J.C. Evaluation of enkephalinase inhibition in the living mouse, using [^{3}H]acetorphan as a probe, *J.Pharmacol.Exp.Ther.*, **1988**, *247*, 653–660.

Acetylcholine



Molecular formula: $C_7H_{16}ClNO_2$ (chloride)
Molecular weight: 181.66 (chloride)
CAS Registry No.: 51-84-3, 60-31-1 (chloride), 66-23-9 (bromide)
Merck Index: 87

SAMPLE

Matrix: CSF, dialysate, tissue
Sample preparation: Dialysate. Inject dialysate directly. Tissue. Homogenize brain tissue with 10 volumes 100 mM perchloric acid (Potter-Elvehjem), let stand on ice for 15 min, centrifuge at 4000 g for 15 min, inject a 0.5 μL aliquot. CSF. Deproteinize by passing through a 0.02 μm Anatop 10 syringe filter (Alltech), inject a 0.5 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 100 × 1 PEEK column packed with Aminex A-9 (Bio-Rad)
Mobile phase: 200 mM pH 8.0 K/Na 3/1 phosphate buffer containing 5 mM NaCl and 0.1% Kathon CG
Column temperature: 25
Flow rate: 0.06 (obtained with a flow splitter)
Injection volume: 0.5
Detector: E, AMOR (Spark Holland), platinum working electrode + 250 mV, carbon composite auxiliary electrode, Ag/AgCl reference electrode, following post-column reaction. The column effluent flowed through a reactor which had 4 U acetylcholine esterase (EC 3.1.1.7 type VI-S from electric eel, 260 IU/mg) and 4 U choline oxidase (EC 1.1.3.17 from Alcaligenes sp., 12.7 IU/mg) enclosed between two 0.01 μm cellulose nitrate filters (Sartorius) (construction details given) to the detector.

CHROMATOGRAM

Retention time: 12
Limit of detection: 10 fmole

OTHER SUBSTANCES

Extracted: choline

KEY WORDS

use metal-free tubing and connectors; solvent reservoir; column; reactor; and detector maintained at 25; rat; brain; human; post-column reaction; immobilized enzyme reactor

REFERENCE

Flentge,F.; Venema,K.; Koch,T.; Korf,J. An enzyme-reactor for electrochemical monitoring of choline and acetylcholine: applications in high-performance liquid chromatography, brain tissue, microdialysis and cerebrospinal fluid, *Anal.Biochem.*, **1992**, *204*, 305–310.

SAMPLE

Matrix: blood
Sample preparation: Add neostigmine and ethylhomocholine to plasma or red blood cells. 150 μL Plasma or red blood cells + 1 mL 400 mM perchloric acid, let stand at 0-4° for 30 min, centrifuge at 5500 g for 1 min. Remove a 750 μL aliquot of the supernatant and add it to 36 μL 10 M potassium acetate, let stand at 0-4° for 5 min, centrifuge at 5500 g for 1 min, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 1.9 Chrompack reversed phase

Column: 100 × 3 Chromspher 5 C18 (Chrompack) (Prepare column by washing with MeOH, MeOH:water 50:50, water, and 5 mg/mL sodium laurylsulfate in water (each wash 20 min at 1 mL/min). Thoroughly wash pump with water (column off line), wash column with water for 5 min and mobile phase for 1 h. Column should be disconnected from the pre-column, reactor, and detector. [Chromatographia, 1987,24,827].)
Mobile phase: 200 mM pH 8.0 potassium phosphate buffer containing 5 mM KCl
Flow rate: 0.6
Injection volume: 100
Detector: E, Spark Holland Amor, Pt working electrode +500 mV, carbon composite auxiliary electrode, Ag/AgCl reference electrode following an enzyme reactor. (Reactor was a 10 × 2.1 Hypersil APS-2 column, activate with glutaraldehyde, equilibrate with mobile phase for 20 min. Inject 80 U acetylcholine esterase (EC 3.1.1.7, type VI-S from electric eel, 260 IU/mg) and 40 U choline oxidase (EC 1.1.3.17 from Alcaligenes sp., 12.7 IU/mg) in 500 μL mobile phase onto the reactor and pump through at 0.05 mL/min for 20 min with mobile phase. [Chromatographia, 1987,24,827])

CHROMATOGRAM
Retention time: 9
Internal standard: ethylhomocholine (5)
Limit of detection: 10 nM

OTHER SUBSTANCES
Extracted: choline

KEY WORDS
plasma; red blood cells; human; mouse

REFERENCE
Damsma,G.; Flentge,F. Liquid chromatography with electrochemical detection for the determination of choline and acetylcholine in plasma and red blood cells. Failure to detect acetylcholine in blood of humans and mice, *J.Chromatogr.*, **1988**, *428*, 1–8.

SAMPLE
Matrix: dialysate
Sample preparation: Inject 10 μL of rat brain dialysate.

HPLC VARIABLES
Guard column: ACH-3-G guard cartridge (ESA)
Column: 150 × 3 5 μm ACH-3 polymeric reversed-phase column (ESA)
Mobile phase: 100 mM sodium phosphate + 0.5 mM tetramethylammonium chloride + 0.005% Reagent MB (a microbicide from ESA) + 2 mM octanesulfonic acid, final pH 8.0
Column temperature: 35
Flow rate: 0.35
Injection volume: 10
Detector: E, ESA Coulochem Model 5200A, Model 5040 analytical cell, palladium reference electrode, stainless steel counter electrode, platinum working electrode + 300 mV following a solid-phase reactor containing immobilized acetylcholinesterase and choline oxidase (reactor temp 35)

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 3 μM

OTHER SUBSTANCES
Extracted: choline

KEY WORDS
brain; rat

REFERENCE
Greaney,M.D.; Marshall,D.L.; Bailey,B.A.; Acworth,I.N. Improved method for the routine analysis of acetylcholine release in vivo: quantitation in the presence and absence of esterase inhibitor, *J.Chromatogr.*, **1993**, *622*, 125–135.

SAMPLE
Matrix: dialysate
Sample preparation: Inject 5 μL aliquots of the dialysate (Ringer's solution).

HPLC VARIABLES
Column: 530 × 1 cation exchange MF-8904 (Bioanalytical Systems)
Mobile phase: 50 mM Na_2HPO_4 containing 5 mL/L Kathon CG (Bioanalytical Systems CF-2150) (Mobile phase was only partially degassed; some oxygen is essential for the enzyme reactor.)
Flow rate: 0.14
Injection volume: 5
Detector: E, Bioanalytical Systems LC-4C, peroxidase-redox polymer coated glassy carbon electrode +100 mV (Anal.Chem. 1992, 64, 3084), Ag/AgCl reference electrode. The column effluent passed through a 50 × 1 immobilized-enzyme reactor containing acetylcholinesterase (EC 3.1.1.7) and choline oxidase (EC 1.1.3.17) (Bioanalytical Systems MF-8903) and flowed to the detector.

CHROMATOGRAM
Retention time: 20
Limit of detection: 10 fmole

OTHER SUBSTANCES
Extracted: choline

KEY WORDS
rat

REFERENCE
Huang,T.; Yang,L.; Gitzen,J.; Kissinger,P.T.; Vreeke,M.; Heller,A. Detection of basal acetylcholine in rat brain microdialysate, *J.Chromatogr.B*, **1995**, *670*, 323–327.

SAMPLE
Matrix: dialysate
Sample preparation: Inject a 10 μL aliquot of dialysate onto a 55 × 1 reactor containing immobilized choline oxidase and catalase (BAS). (The enzymes destroy choline but not acetylcholine. Acetylcholine is eluted from the reactor onto the analytical column.)

HPLC VARIABLES
Column: 530 × 1 10 μm ACh (BAS)
Mobile phase: 28 mM pH 8.5 Na_2HPO_4 containing 0.5% of 1% Kathon antimicrobial solution (BAS)
Flow rate: 0.1
Injection volume: 10
Detector: E, Pt electrode +0.5 V, Ag/AgCl reference electrode following post-column reaction. The column effluent flowed through a 50 × 1 reactor packed with immobilized acetylcholine oxidase and choline oxidase (BAS) to the detector. (Acetylcholine was converted to hydrogen peroxide which was then detected.)

CHROMATOGRAM
Retention time: 13
Limit of detection: 5 nM
Limit of quantitation: 10 nM

KEY WORDS
post-column reaction; rat; immobilized enzyme reactor

REFERENCE
Tsai,T.-R.; Cham,T.-M.; Chen,K.-C.; Chen,C.-F.; Tsai,T.-H. Determination of acetylcholine by on-line microdialysis coupled with pre- and post-microbore column enzyme reactors with electrochemical detection, *J.Chromatogr.B*, **1996**, *678*, 151–155.

SAMPLE
Matrix: formulations
Sample preparation: Make up the lyophilized preparation in sterile water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: Mix (? g) sodium 1-heptanesulfonate (Waters PIC Reagent B-7) in 900 mL water, adjust pH to 4.0 with 6 M ammonium hydroxide, add 50 mL MeCN, make up to 1 L with water
Flow rate: 2
Injection volume: 50
Detector: RI

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: choline
Noninterfering: acetic acid, mannitol

KEY WORDS
stability-indicating

REFERENCE
Tao,F.T.; Thurber,J.S.; Dye,D.M. High-performance liquid chromatographic determination of acetylcholine in a pharmaceutical preparation, *J.Pharm.Sci.*, **1984**, *73*, 1311–1313.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in saline, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:buffer 10:90 (Buffer was 20 mL Low-UV PIC B-7 (Waters) diluted with 480 mL water (10 mM 1-heptanesulfonic acid).)
Flow rate: 1
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 12.6

REFERENCE
Woodman,T.F.; Johnson,B.; Marwaha,R.K. Determination of methacholine chloride by ion-pair high-pressure liquid chromatography, *J.Liq.Chromatogr.*, **1982**, *5*, 1341–1348.

SAMPLE
Matrix: solutions

Sample preparation: solutions

HPLC VARIABLES

Column: 30 × 2.1 Aquapore AX300 (Brownlee) (Prepare the column by injecting ten 20 μL aliquots of 10 mg/mL choline oxidase (from Alcaligenes sp, 33 U/mg) and 100 μL cholinesterase (Type III, from electric eel, 970 U/mg, 0.65 mg/mL). Replenish enzymes every 1-2 weeks. Acetylcholine is converted to choline and choline is converted to hydrogen peroxide.)
Mobile phase: 20 mM pH 7 Tris-acetate buffer containing 1 mM tetramethylammonium chloride and 200 μM octanesulfonate
Flow rate: 2
Detector: E, BAS LC4B, Pt working electrode +0.5 V, Ag/AgCl reference electrode

KEY WORDS

post-column reaction; immobilized enzyme reactor

REFERENCE

Meek,J.L.; Eva,C. Enzymes adsorbed on an ion exchanger as a post-column reactor: application to acetylcholine measurement, *J.Chromatogr.*, **1984**, *317*, 343–347.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 4 mM solution in water, inject a 10 μL aliquot

HPLC VARIABLES

Column: μBondapak C18 Radial-Pak in a RCM-100 radial compression module
Mobile phase: Butanol:MeOH:acetic acid:water 8:4:2:86 containing 0.15 mM 1-phenethyl-2-picolinium bromide (Extract 10 mM 1-phenethyl-2-picolinium bromide stock solution with dichloromethane before use to remove UV-absorbing impurities.)
Flow rate: 3
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 3

OTHER SUBSTANCES

Simultaneous: choline, butyrylcholine, propionylcholine

REFERENCE

Jones,R.S.; Stutte,C.A. Chromatographic analysis of choline and acetylcholine by UV visualization, *J.Chromatogr.*, **1985**, *319*, 454–460.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in n-propanol:water 80:20 or DMF:water 80:20, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrospher 100 Diol
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in MeCN. B was 0.1% trifluoroacetic acid in water. A:B 90:10 for 1 min, to 70:30 over 17 min, to 0:100 over 2 min, maintain at 0:100 over 4.5 min
Flow rate: 0.9
Detector: Evaporative light scattering (Sédex 55, Sédéré)

CHROMATOGRAM

Retention time: 3.85

OTHER SUBSTANCES
Simultaneous: choline, sodium, magnesium, calcium

REFERENCE
Treiber,L.R. Normal-phase high-performance liquid chromatography with relay gradient elution. I. Description of the method, *J.Chromatogr.A*, **1995**, *696*, 193–199.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize brain tissue with 3 mL 400 mM perchloric acid containing 2 nmoles ethylhomocholine, centrifuge at 35000 g for 20 min, adjust pH of supernatant to about 4.2 with about 200 μL 7.5 M potassium acetate, centrifuge at 35000 g for 20 min. Add the supernatant to 100 μL 5 mM tetramethylammonium chloride, add 3 mL 2% ice-cold reineckate solution, let stand on ice for 1 h, centrifuge at 1000 g at 0° for 10 min. Remove the supernatant and dry the precipitate under vacuum overnight, add about 1 mL 5 mM silver tosylate in MeCN until the pink color disappears, centrifuge at 1000 g at 0° for 10 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL 20 mM pH 3.5 citrate-phosphate buffer, inject an aliquot. Alternatively (for acetylcholinme only), homogenize brain tissue with 400 μL 20 mM pH 3.5 citrate-phosphate buffer, add 4.8 nmoles ethylhomocholine, boil for 10 min, add 40 μL 250 mM zinc sulfate while vortexing, add 40 μL 250 mM barium hydroxide while vortexing, centrifuge at 35000 g for 15 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: ODS-5 (Bio-Rad)
Column: 150 mm long Bio-Sil ODS-5S (Bio-Rad)
Mobile phase: Buffer (Buffer was 10 mM sodium acetate buffered to pH 5 with 20 mM citric acid, containing 4.5 mg/L sodium octyl sulfate and 1.2 mM tetramethylammonium chloride.)
Flow rate: 0.8
Injection volume: 20
Detector: E, Bio Analytical Systems LC-4A, Pt electrode +0.5 V, Ag/AgCl reference electrode following post-column reaction detection. The column effluent mixed with 1 U/mL choline oxidase and 2 U/mL acetylcholinesterase in 200 mM pH 8.5 phosphate buffer pumped at 0.5 mL/min, the mixture flowed through a 30 m × 0.3 mm i.d. PTFE tube (2.5 min) to the detector

CHROMATOGRAM
Retention time: 10
Internal standard: ethylhomocholine (7.2)
Limit of detection: 1 pmole

OTHER SUBSTANCES
Extracted: choline

KEY WORDS
post-column reaction; rat; brain

REFERENCE
Potter,P.E.; Meek,J.L.; Neff,N.H. Acetylcholine and choline in neuronal tissue measured by HPLC with electrochemical detection, *J.Neurochem.*, **1983**, *41*, 188–194.

SAMPLE
Matrix: tissue
Sample preparation: Sonicate 250 mg rat brain tissue with 6 mL 1 M formic acid containing 10 nmoles IS for 5 min, centrifuge at 4° at 10000 g for 20 min, add the supernatant

to an equal volume of diethyl ether, add 5 mL water, shake, centrifuge at 2000 g for 5 min, discard the organic layer. Lyophilize the aqueous layer, dissolve the residue in 400 μL water, filter (0.45 μm). Add 30 μL reagent to the filtrate, mix, centrifuge at 10000 g for 5 min. Dissolve the precipitate in 300 μL water, add 50 mg Dowex 1x8, shake, centrifuge at 10000 g for 5 min, inject a 10 μL aliquot of the supernatant. (Reagent contained 20% KI and 18% iodine in water.)

HPLC VARIABLES

Column: 150 × 4.6 Nucleosil C18

Mobile phase: Buffer (Prepare buffer by dissolving 1.36 g sodium acetate. 3.72 disodium EDTA, 25 mg sodium octyl sulfate, and 1.2 mmoles tetramethylammonium chloride in 900 mL water, adjust pH to 5.0 with 200 mM citric acid, make up to 1 L.)

Column temperature: 37

Flow rate: 0.8

Injection volume: 10

Detector: E, Bioanalytical Systems LC-4B/17, TL-10A platinum electrode +500 mV, Ag/AgCl reference electrode following post-column reaction. The effluent from the column mixed with buffer pumped at 0.5 mL/min and the mixture flowed through an immobilized enzyme reactor to the detector. (Prepare buffer by dissolving 71.6 g $Na_2HPO_4.12H_2O$ and 372 mg disodium EDTA in 900 mL water, adjust pH to 8.5 with NaH_2PO_4, make up to 1 L. Prepare reactor by heating 200-400 mesh porous glass beads (pore size 400 Å, Electronucleonics, Fairfield NJ) in 5% nitric acid, wash with water, dry, add to 10% 3-aminopropyltriethoxysilane in toluene, reflux overnight. Suspend the beads in 2% glutaraldehyde in water at room temperature for 2 h. Dissolve 0.45 mg acetylcholinesterase (type III, EC.3.1.1.7, Sigma) and 16.6 mg choline oxidase (EC.1.1.3.17, Sigma) in 200 μL 50 mM pH 7.0 phosphate buffer containing 10 mM disodium EDTA, add 500 mg activated beads, pack in a 10 × 4 tube.)

CHROMATOGRAM

Retention time: 10

Internal standard: ethylhomocholine (Prepare ethylhomocholine by adding 3-dimethylamino-1-propanol to EtOH, add bromoethane. When reaction is complete add ether, filter off the precipitate and wash it with ether.) (8)

Limit of detection: 100 fmoles

OTHER SUBSTANCES

Extracted: choline

KEY WORDS

post-column reaction; rat; brain; immobilized enzyme reactor

REFERENCE

Asano,M.; Miyauchi,T.; Kato,T.; Fujimori,K.; Yamamoto,K. Determination of acetylcholine and choline in rat brain tissue by liquid chromatography/electrochemistry using an immobilized enzyme post column reactor, *J.Liq.Chromatogr.*, **1986**, *9*, 199–215.

SAMPLE

Matrix: tissue

Sample preparation: Sonicate rat brain with ten volumes of 1 M formic acid:acetone 15:85 containing IS, centrifuge at 4° at 20000 g. Remove a 500 μL aliquot of the supernatant and add it to 2 mL heptane:chloroform 80:10, vortex. Remove the aqueous layer and add it to 250 μL 3 mg/mL sodium tetraphenylboron in 3-heptanone, vortex. Remove a 200 μL aliquot of the upper organic layer and add it to 50 μL 1 M HCl, vortex. Remove the aqueous layer and evaporate it to dryness under reduced pressure, reconstitute with mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES

Guard column: C18 (Waters)

Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: 100 mM pH 7 KH_2PO_4 containing 10 μg/mL sodium octane sulfate and 600 μg/mL tetramethylammonium chloride
Flow rate: 1
Injection volume: 30
Detector: E, Chromatofield, Pt electrode +0.5 V following post-column reaction. The column effluent flowed through an immobilized enzyme reactor to the detector. (Prepare reactor by heating 200-400 mesh porous glass beads (pore size 350 Å, Sigma) in 5% nitric acid at 100° for 1 min, wash with water, dry, add to 10% 3-aminopropyltriethoxysilane in toluene, heat at 115° for 12 h. Suspend the beads in 2% glutaraldehyde in water at room temperature for 2 h. Dissolve 100 U acetylcholinesterase (type III, electric eel, Sigma) and 100 U choline oxidase (Alcaligenes, Sigma) in 1 mL 50 mM pH 7 phosphate buffer, add 120 mg activated beads, shake periodically, pack in a 20 × 2 tube.)

CHROMATOGRAM

Retention time: 9.5
Internal standard: ethylhomocholine bromide (Prepare ethylhomocholine by adding 3-dimethylamino-1-propanol to EtOH, add bromoethane, when reaction is complete add ether, filter off the precipitate and wash it with ether.) (7)

OTHER SUBSTANCES

Extracted: choline

KEY WORDS

rat; brain; post-column reaction; immobilized enzyme reactor

REFERENCE

Beley,A.; Zekhnini,A.; Lartillot,S.; Fage,D.; Bralet,J. Improved method for determination of acetylcholine, choline, and other biogenic amines in a single brain tissue sample using high performance liquid chromatography and electrochemical detection, *J.Liq.Chromatogr.*, **1987**, *10*, 2977–2992.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize brain tissue with 1 mL 50 mM perchloric acid containing 10 nmoles ethylhomocholine for 1 min (Nissei Model US-300T, 300 W, 20 kHz), centrifuge at 20000 g at 4° for 15 min, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 10 × 4 glassy carbon particles IRICA Type CP-2250 (IRICA Instruments) (removes interfering catecholamines but is not essential)
Column: 60 × 4 3 μm Acetylcholine Separation polymeric styrene-based column (Bioanalytical Systems)
Mobile phase: 50 mM pH 8.4 phosphate containing 1 mM disodium EDTA and 0.4 mM sodium 1-octanesulfonate
Column temperature: 35
Flow rate: 0.8
Injection volume: 20
Detector: E, Bioanalytical systems LC-4A, dual platinum electrodes + 0.50 V, Ag/AgCl reference electrode, following a 5 × 4 reactor containing immobilized acetylcholinesterase and choline oxidase

CHROMATOGRAM

Retention time: 7.8
Internal standard: ethylhomocholine (4.07)

OTHER SUBSTANCES

Extracted: choline

KEY WORDS
rat; brain

REFERENCE
Ikarashi,Y.; Iwatsuki,H.; Blank,C.L.; Maruyama,Y. Glassy carbon pre-column for direct determination of acetylcholine and choline in biological samples using liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1992**, *575*, 29–37.

SAMPLE
Matrix: tissue
Sample preparation: Mix brain or heart tissue with 2 mL 6% trichloroacetic acid, homogenize for 10 min at 0°, let stand in an ice bath for 10 min, add 2 mL 100 mM pH 7.4 sodium phosphate, mix, let stand in an ice bath for 10 min, centrifuge at 30000 g for 25 min, dilute the supernatant ten-fold with 100 mM pH 7.4 sodium phosphate, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelguard LC-8
Column: 250 × 4 5 μm Supelcosil LC-8
Mobile phase: 100 mM pH 7.4 phosphate buffer containing 4 mM tetramethylammonium perchlorate and 0.1 mM EDTA
Flow rate: 0.5
Injection volume: 50
Detector: E, Biometra Model EP 30, 0.45 V following a 30 × 2.1 cartridge with immobilized acetylcholinesterase and cholinoxidase (Biometra)

CHROMATOGRAM
Retention time: 14
Limit of detection: <5 pmole

KEY WORDS
brain; heart

REFERENCE
Salamoun,J.; Nguyen,P.T.; Remien,J. Cation-exchange liquid chromatography of choline and acetylcholine on free shielded silanols of silica-based reversed-phase stationary phases, *J.Chromatogr.*, **1992**, *596*, 43–49.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize brain tissue with 10 volumes 400 mM perchloric acid, centrifuge. Remove the supernatant and add it to one tenth the volume of 7.5 M potassium acetate solution, centrifuge. Remove a 100 μL aliquot and take it to dryness in a vacuum centrifuge, dissolve the residue in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 60 × 4.6 5 μm Bakerbond Sulfopropyl
Mobile phase: 100 mM pH 7.5 sodium phosphate with 5 mM tetramethylammonium chloride
Flow rate: 1.2
Detector: E, Biometra EP 20, platinum electrode + 0.5 V following an immobilized enzyme reactor containing choline oxidase (EC 1.1.3.17) and acetylcholine esterase (EC 3.1.1.7) to convert acetylcholine and choline to hydrogen peroxide which was then detected

CHROMATOGRAM
Retention time: 3.8
Limit of detection: 0.3 pmol

OTHER SUBSTANCES
Extracted: choline

KEY WORDS
rat; brain

REFERENCE
Klein,J.; Gonzalez,R.; Köppen,A.; Löffelholz,K. Free choline and choline metabolites in rat brain and body fluids: sensitive determination and implications for choline supply to the brain, *Neurochem.Int.*, **1993**, *22*, 293–300.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Nissei US-300T ultrasonic cell disrupter at 300 W and 20 kHz for 1 min) rat brain striatal tissue with 1 mL 1 μM ethylhomocholine in 50 mM perchloric acid, centrifuge at 4° at 20000 g for 15 min, filter (0.45 μm) the supernatant, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4 74-149 μm plastic formed carbon (details in paper)
Column: 60 × 4 3 μm Acetylcholine Separation polymeric styrene column (Bioanalytical Systems)
Mobile phase: 50 mM pH 8.40 Phosphate buffer containing 1 mM disodium EDTA and 0.40 mM sodium 1-octanesulfonate
Column temperature: 35 ± 1
Flow rate: 0.7
Injection volume: 10
Detector: E, Bioanalytical Systems LC-4A, dual Pt working electrode +500 mV, Ag/AgCl reference electrode following post-column reaction. The effluent from the column flowed through a 5 × 4 immobilized enzyme reactor containing acetylcholinesterase and choline oxidase (Bioanalytical Systems) to the detector.

CHROMATOGRAM
Retention time: 12.5
Internal standard: ethylhomocholine (6.5)

OTHER SUBSTANCES
Extracted: choline
Noninterfering: 3,4-dihydroxybenzylamine, dopamine, norepinephrine

KEY WORDS
rat; brain; guard-column removes interferences from catecholamines; post-column reaction; immobilized enzyme reactor

REFERENCE
Ikarashi,Y.; Blank,C.L.; Suda,Y.; Kawakubo,T.; Maruyama,Y. Application of a novel, plastic formed carbon as a precolumn packing material for the liquid chromatographic determination of acetylcholine and choline in biological samples, *J.Chromatogr.A*, **1995**, *718*, 267–272.